Fiscal Year:	FY 2018	**Task Last Updated:**	FY 10/29/2018
PI Name:	Kabarowski, Janusz Ph.D.		
Project Title:	Radiation-Induced Early Changes in Gene and Protein Expression, Lipid Oxidative States, and Vascular Function Related to Atherosclerosis		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **Cardiovascular**:Risk of Cardiovascular Adaptations Contributing to Adverse Mission Performance and Health Outcomes		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	35294	**Congressional District:**	7
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No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA JSC
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	January 2017: New Principal Investigator is Dr. Janusz Kabarowski; Previous PI was Dr. Dennis Kucik.		
COI Name (Institution):	White, Charles Roger Ph.D. (University of Alabama, Birmingham)		
Grant/Contract No.:	NNX14AH88G		
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Performance Goal Text:			

Task Description:	NOTE: Continues "Radiation-Induced Early Changes in Gene and Protein Expression, Lipid Oxidative States, and Vascular Function Related to Atherosclerosis" with Principal Investigator (PI) Dr. Dennis Kucik. Dr. Janusz Kabarowski is new PI as of January 1, 2017, due to Dr. Kucik's retirement. Epidemiological studies have established that radiation from a number of terrestrial sources increases the risk of atherosclerosis, even at relatively low doses. Although the heavy ions found in galactic cosmic radiation (GCR) interact very differently with tissues than do the high- energy photons that comprise most terrestrial radiation, it has been thought that GCR could pose a risk as well. In our previous project, we showed that heavy ions such as 0Fe and 28Si are indeed atherogenic, accelerating the development of plaques in the apoE -/- mouse model. Mouse models, however, are known (from comparison of animal experiments to human epidemiological data) to require much higher radiation doses for plaque formation than humans. This is likely due to species differences in lipid metabolism and the immune system. Plaque formation is a very late endpoint, however. While species differences may lead to divergence in pathological consequences at times distant from absorption of radiation, very early steps, such as changes in gene expression, protein expression, and lipid oxidation, are likely to be similar in humans and mice. Thus, analysis of these early changes in response to space-relevant doses of 56Fe and protons will enable us to better predict risk of cardiovascular consequences from space radiation and increase our understanding of the mechanisms involved. In addition, a focus on these early events will obviate the need for models incorporating unnaturally high plasma lipid and cholesterol levels, so that healthy astronauts can be more closely simulated. Our hypothesis is that irradiation of blood vessels in vivo leads to early species-independent changes in endothelial cell genes, proteins and lipids that elicit vascular dysfunction and are therefore likely to be predictive of cardiovascular disease. An understanding of these changes can be used to help predict risk and propose countermeasures. We will test this hypothesis through completion of the following specific aims: Aim 1: Determine, as a function of radiation dose, acute, radiation-induced atherosclerosisassociated changes in gene and protein expression in aortic endothelial cells of irradiated mice. Aim 2: Determine, as a function of radiation dose, changes in oxidation states of aortic endothelial cell lipids. Aim 3: Determine the dose dependence of functional consequences of 56Fe, and protons with respect to perturbations in endothelial reactivity and aortic adhesivity. Aim 4: Develop a systems biology model of response of aortic endothelial cells to radiation damage to determine molecular mechanisms.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Atherosclerosis is a major adverse effect of therapeutic radiation. It is not always possible to avoid exposure of the heart and major arteries, especially for treatment of head-and-neck cancers and breast cancer. This project will provide important new information about the mechanism of radiation-induced atherosclerosis to help in designing possible mitigating strategies. Moreover, the use of ion beams for cancer treatment is becoming ever more prevalent. In the U.S., proton therapy is becoming more widespread, and carbon-ion therapy is available in Europe and Asia. Little is known about the cardiovascular effects of these new radiation treatment modalities. This work will greatly enhance our understanding of possible adverse effects.
	SUPPLEMENTAL REPORTING FOR FINAL REPORT -- OCTOBER 2018 Task Progress: We have arranged our results in terms of progress on Roadmap questions below. Degen-1, “How can tissue specific risk models be developed for the major degenerative tissue risks, including heart, circulatory, endocrine, digestive, lens, and other tissue systems in order to estimate GCR and SPE (solar particle events) risks for degenerative diseases?” Using an ex vivo experimental system for aortic monocyte adhesion that we developed, we determined last year that increased vascular adhesion is a major early event following exposure to space relevant doses of 56Fe radiation. We also determined by pertussis toxin incubation of monocytes prior to addition to ex vivo aortic preparations from control and 56Fe irradiated C57Bl/6J mice that this adhesive effect requires signaling from Gi class of G proteins to which chemokine receptors typically signal to mediate firm adhesion of leukocytes to activated endothelium. Using a cytokine/chemokine array, we were also able to identify chemokines that are elevated in the blood of mice after exposure to 56Fe. These may prove to be useful biomarkers for cardiovascular risk prediction. In the final period of funding, we have completed analysis of potential effects of protons on atherosclerosis in the hyperlipidemic Apolipoprotein E knockout (ApoE-/-) mouse model and subsequently analyzed possible effects of the same dose of protons on aortic adhesivity in normal mice. Unlike exposure to 56Fe, which we have previously shown to accelerate atherosclerosis in ApoE-/- mice and promote adhesivity of aortae from C57BL/6J mice, protons had no such effect; atherosclerosis in ApoE-/- mice was not accelerated even by doses of 2, 5, or 10 Gy protons and aortae from C57BL6/J mice exposed to the same proton dose showed no sign of increased adhesivity. These results not only support the notion that protons may not be as damaging for the cardiovascular system compared to heavy ions like 56Fe, but also validate (due to the co-penetrance of atherosclerosis and adhesive phenotypes in 56Fe or proton exposed mouse models) the aortic adhesion model we developed in previous years of this grant’s funding [Ed. note: see also reports uner Principal Investigator Dennis Kucik] as a tool for future studies aimed at quantifying risk for radiation-induced vascular effects related to atherosclerosis. These future studies must also involve assessment of latency of these adhesive effects following radiation, and whether such vascular changes do indeed contribute over time to accelerating atherosclerosis. This will be accomplished by “switching” radiation exposed mice at various time-points to a hyperlipidemic state through the injection of a liver-tropic adeno-associated vector (AAV) encoding a gain-of-function mutant (D377Y) of Pro-protein Convertase Subtilsin-Kexin type 9 (PCSK9) capable of inhibiting endogenous liver LDL receptors to induce a state of hyperlipidemia similar to that in commonly used atherosclerosis models like the LDL receptor or ApoE knockout mice. By employing such a method, one obviates the need for using standard ApoE or LDL receptor knockout mouse models harboring extreme hyperlipidemia with no relevance to normal human physiology that could affect vascular and other responses during and following radiation exposure, but still allowing measures of atherosclerosis to be performed by “switching on” a hyperlipidemic pro-atherogenic state at later times to assess atherosclerotic lesion formation.

Task Progress:

Degen-2, “What are the mechanisms of degenerative tissues risks in the heart, circulatory, endocrine, digestive, lens, and other tissue systems? What surrogate endpoints do they suggest?”

To understand how early vascular adhesive effects of 56Fe radiation are mediated in the vascular tissue and to identify biomarkers for better risk prediction and countermeasure development, we have previously completed analyses of lipids in the aorta of mice 6 weeks after exposure to space relevant doses of 56Fe (a time-point at which aortic adhesion is maximal). The numbers of lipids changed in the aortae by 56Fe exposure of the mice depended on the dose of radiation and several important lipid classes already linked to vascular inflammation and/or atherosclerosis were elevated, including sphingomyelins that are known to have inflammatory effects on smooth muscle cells and/or other vascular cell types, and ceramides that may be associated with vascular cell death. Most interestingly in terms of possible countermeasures to protect against oxidation related injury caused by space radiation exposure, ether-linked phosphatidylcholines were elevated within 6 weeks of 56Fe exposure; these lipids are thought to have antioxidant properties and are thus excellent candidates for countermeasure development in the future. Analysis of the plasma lipidome revealed that only 10 lipid species changed following exposure to the same 56Fe doses and time-points employed in the aortic lipidomic analyses. All changes were reductions in abundance. These lipids included N-acylphosphoethanolamines (NAPEs), which are members of the endocannabinoid system, as well as SMs. Most interestingly, ether-linked phosphatidylethanolamines (PEs), structurally related to the ether-linked PCs increased in aortae at the same time-point and similarly having potential anti-oxidant function, were among the lipids decreased in the plasma. This suggests that these potentially anti-oxidant ether-linked PEs may be consumed following total body exposure to 56Fe and may thus serve as a possible biomarker for atherosclerosis risk assessment. We also completed a genomics analysis of gene expression changes in endothelium enriched aortic tissue from 56Fe exposed C57Bl/6J mice in which the adhesive effects of the same 56Fe radiation doses were observed at the 6 weeks time-point. Genomics was performed by Next-Generation sequencing on Illumina Platforms. Several genes with potential roles in modifying endothelial function were among the most downregulated following 56Fe exposure, including CHST4, a sulfotransferase involved in O-glycosylation of selectins, and also a major Transient Receptor Potential Canonical channel receptor, TRPC6, that not only regulates endocannabinoid signaling, but may also function to control transendothelial migration.

The last period of funding (July 2017-July 2018) has been a one year no-cost extension of the grant following the retirement of the original PI. This enabled Dr. Kabarowski (the new PI) to complete all necessary data analyses and lipidomics experiments in preparation for publication. We have also completed in this time a new aortic lipidomic analysis for the 2 weeks time-point following exposure of mice to the same doses of 56Fe as in our 6 weeks time-point experiment. Fewer lipids are changed at this earlier time-point and currently we are completing the data analysis for each individual lipid species.

These results constitute progress on Aim #2: Determine, as a function of radiation dose, changes in oxidation states of aortic endothelial cell lipids, and demonstrates that 56Fe radiation causes changes in vascular and blood lipids that may predict atherosclerotic disease risk and/or progression.

OCTOBER 2017 REPORT:

Task Progress: We have arranged our results from this year of funding in terms of progress on Roadmap questions below.

Degen-1, “How can tissue specific risk models be developed for the major degenerative tissue risks, including heart, circulatory, endocrine, digestive, lens and other tissue systems in order to estimate GCR and SPE (solar particle events) risks for degenerative diseases?”

Using an ex vivo experimental system for aortic monocyte adhesion that we developed, we determined last year that increased vascular adhesion is a major early event following exposure to space relevant doses of 56Fe radiation. We also determined by pertussis toxin incubation of monocytes prior to addition to ex vivo aortic preparations from control and 56Fe irradiated C57Bl/6J mice that this adhesive effect requires signaling from Gi class of G proteins to which chemokine receptors typically signal to mediate firm adhesion of leukocytes to activated endothelium. Using a cytokine/chemokine array, we were also able to identify chemokines that are elevated in the blood of mice after exposure to 56Fe. These may prove to be useful biomarkers for cardiovascular risk prediction.

Degen-2, “What are the mechanisms of degenerative tissues risks in the heart, circulatory, endocrine, digestive, lens, and other tissue systems? What surrogate endpoints do they suggest?” To understand how early vascular adhesive effects of 56Fe radiation are mediated in the vascular tissue and to identify biomarkers for better risk prediction and countermeasure development, we have previously completed analyses of lipids in the aorta of mice 6 weeks after exposure to space relevant doses of 56Fe (a time-point at which aortic adhesion is maximal). The numbers of lipids changed in the aortae by 56Fe exposure of the mice depended on the dose of radiation and several important lipid classes already linked to vascular inflammation and/or atherosclerosis were elevated, including sphingomyelins that are known to have inflammatory effects on smooth muscle cells and/or other vascular cell types, and ceramides that may be associated with vascular cell death. Most interestingly in terms of possible countermeasures to protect against oxidation related injury caused by space radiation exposure, ether-linked phosphatidylcholines were elevated within 6 weeks of 56Fe exposure; these lipids are thought to have antioxidant properties and are thus excellent candidates for countermeasure development in the future.

We have now completed analysis of the plasma lipidome, revealing that only 8 lipid species changed following exposure to the same 56Fe doses and time-points employed in the aortic lipidomic analyses. All changes were reductions in abundance. These lipids included N-acylphosphoethanolamines (NAPEs), which are members of the endocannabinoid system, as well as SMs. Most interestingly, ether-linked phosphatidylethanolamines (PEs), structurally related to the ether-linked PCs increased in aortae at the same time-point and similarly having potential anti-oxidant function, were among the lipids decreased in the plasma. This suggests that these potentially anti-oxidant ether-linked PEs may be consumed following total body exposure to 56Fe and may thus serve as a possible biomarker for atherosclerosis risk assessment.

We have now also completed a genomics analysis of gene expression changes in endothelium enriched aortic tissue from 56Fe exposed C57Bl/6J mice in which the adhesive effects of the same 56Fe radiation doses were observed at the 6 weeks time-point. Genomics was performed by Next-Generation sequencing on Illumina Platforms. Several genes with potential roles in modifying endothelial function were amongst the most downregulated following 56Fe exposure, including CHST4, a sulfotransferase involved in O-glycosylation of selectins, and also a major Transient Receptor

	Potential Canonical channel receptor, TRPC6, that not only regulates endocannabinoid signaling, but also functions to control transendothelial migration. These results constitute progress on Aim #2: Determine, as a function of radiation dose, changes in oxidation states of aortic endothelial cell lipids, and demonstrates that 56Fe radiation causes changes in vascular and blood lipids that may predict atherosclerotic disease progression.
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